

Electric Times in Olfaction

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Recent work established the spread of interglomerular excitation in the *Drosophila* antennal lobe. Two papers in this issue of *Neuron*, by Huang et al. and Yaksi and Wilson, show that cholinergic *krasavietz* local interneurons are a major substrate for this spread of excitation, predominantly via electrical coupling.

Understanding systems of neurons in action remains one of the central problems of neuroscience. Olfactory circuits, with their exquisitely well described molecular architecture and their similar designs across many species, offer a rare opportunity to address some of these complex systems issues. The olfactory system of the fruit fly *Drosophila melanogaster*, with its small size and genetic manipulability, make it particularly useful (Borst, 1983; Clyne et al., 1999; Vosshall et al., 1999). The fly has two sets of olfactory organs: the antennae and the maxillary palps. A total of ~2500 olfactory receptor neurons (ORNs) innervate these organs and provide the fly with its odor sensitivity. Most ORNs express one of ~45 olfactory receptor proteins, and all ORNs also express *Or83b*, which seems to be required for cellular housekeeping. ORN axons project bilaterally to spherical bundles of neuropil called glomeruli, and each glomerulus receives ORNs expressing the same set of ORs. The ~50 glomeruli in each hemisphere are packed tightly together to form the antennal lobes (AL). In addition to ORNs, each glomerulus is innervated by an average of three projection neurons (PNs), each of which contacts all of the ORN axons innervating the glomerulus. Almost all PNs are excitatory and innervate a single glomerulus (a few inhibitory multiglomerular PNs exist). Each AL also contains ~200 local interneurons (LNs), most of which are inhibitory and which tend to innervate many glomeruli. PNs form the sole output of the AL and project to the mushroom body and the lateral horn. The mushroom body plays a central role in learning and memory, while the lateral horn remains poorly understood (Masse et al., 2009; Chou et al., 2010).

Many key questions about olfaction concern the transformations accomplished at each “relay” in the pathway between transduction and behavior. The AL’s principal neurons (the PNs) in particular have been the subject of much interest, for their odor tuning is broad (locusts: Perez-Orive et al., 2002; fruit flies: Wilson et al., 2004; Shang et al., 2007), broader than the specificity of ORN and PN glomerular projections might naively suggest (in flies at least) and indeed broader than that of their cognate ORNs (Wilson et al., 2004; Olsen et al., 2007). Because PNs are mostly uniglomerular, this finding suggested excitatory “lateral” interactions between glomeruli. This was initially perplexing because excitatory interneurons were unknown in this system and the dominant (yet largely unsubstantiated) model of interglomerular interaction was one of “lateral inhibition” à la retina, subserving a putative narrowing of PN tuning. Shortly thereafter, one study in *Drosophila* showed that LNs are not all GABAergic (Wilson and Laurent, 2005) and another that those GABA-immunonegative LNs are choline-acetyl-transferase-positive (ChAT⁺; Shang et al., 2007). Subsequent imaging and electrophysiological studies established the existence of lateral excitation directly by showing that (1) surgically or genetically deafferented PNs are still broadly excited by odors (Olsen et al., 2007; Shang et al., 2007) and that (2) the PN population can still be excited by a range of odors even with only a single functional ORN type (Olsen et al., 2007). A screen for ChAT-positive LNs revealed ~10–15 cells that express *krasavietz-Gal4* and arborize broadly in the AL (Shang et al., 2007). Hence the stage was set to determine systematically

whether these LNs could be the neural substrate for this excitation.

In the fly AL, local spread of excitation has a number of electrophysiological signatures. It broadens the tuning of PNs relative to that of their cognate ORNs, is fast enough that the odor responses of deafferented PNs are not significantly slower than controls, and is nonuniform among PNs, though stereotyped across flies (Olsen et al., 2007). A mechanistic explanation for such a spreading of excitation must explain these observations in terms of neural interactions. Two new papers in this issue of *Neuron* (Huang et al., 2010; Yaksi and Wilson, 2010) now present a compelling case that cholinergic *krasavietz* excitatory LNs are if not the, at least a major part of the neural substrate for lateral excitation, through a widespread yet ordered set of electrical and chemical connections.

Excitatory *krasavietz* LNs (eLNs) were identified using a combination of molecular, anatomical, and electrophysiological criteria—intrinsic responses to current injection in Huang et al.’s study, characteristic synaptic background in both studies. Paired recordings from eLN/PN pairs showed that depolarization and hyperpolarization can be transmitted in either direction, suggesting electrical synapses. Such coupling was found in every pair tested, implying all-to-all connectivity. Although the strength of the connection varied with each pair, electrical coupling was, for each pair, stronger from eLNs to PNs than in the converse direction, consistent with rectification. Since *krasavietz* LNs express ChAT, eLN output synapses to PNs could have a chemical cholinergic component. There is indeed an α -bungarotoxin-sensitive component to the EPSP (both

studies), but its size and relevance is considered minor, or possibly due to spill-over, in Yaksi and Wilson's study. PNs, by contrast, do make excitatory chemical synapses onto eLNs. These findings already provide a potential neural substrate both for the spread of excitation between glomeruli and for a degree of glomerular specificity. Using paired recordings between eLNs, Huang et al. show that they are densely and reciprocally interconnected, and use the statistics of response latency to electrical antennal stimulation to argue that ORN input to eLNs is direct. These latter results help explain the speed of lateral excitation. The case for electrical coupling is strengthened by Yaksi and Wilson's evidence that transmission at putative gap junctions is abolished in *shakB*² mutant flies and by Huang et al.'s anatomical evidence for (asymmetric) dye coupling.

Lateral excitation manifests itself functionally as a broad excitation of PNs by odors, even when PNs are surgically or genetically deafferented. This excitation is powerful and remains with minor decrement throughout the odor presentation (Olsen et al., 2007). Given the dense interconnectivity of PNs and eLNs, this pattern of excitation could be delivered to PNs by eLNs in at least two ways. (1) Each eLN could itself be robustly excited by many or most odors/ORNs, and transmit this excitation to other eLNs and PNs. (2) Alternatively, individual eLNs could have selective and patterned odor responses, but together influence PNs strongly and broadly despite inter-cell variations in responses. While both groups found that eLNs have broad odor responses, Yaksi and Wilson found the responses to be quite uniform in intensity and patterning, even with odors with widely varying effects on the ORN population; Huang et al. by contrast, found eLN responses to odors to be more selectively patterned. The reason for this discrepancy is not clear; both groups recorded odor responses in intact flies, used overlapping criteria for identifying eLNs, and deployed overlapping odor sets for stimulation. Different access routes for recording, odor concentrations or sampling of different eLN subpopulations are reasonable explanations for these differences.

The two groups also investigated interactions of eLNs with traditional, inhibitory (GABA⁺) local interneurons (iLNs). This was prompted by several observations. First many iLNs, like eLNs, arborize broadly throughout the AL, making interactions plausible. Second, *krasavietz* eLNs are ChAT positive, suggesting that they might form with iLNs the cholinergic excitatory synapses that seem weak or elusive in recording from PNs. Finally, both groups observed spontaneous IPSPs in eLN membrane potential recordings, suggestive of chemical inhibitory input. Hence, eLN interactions with iLNs seemed likely and paired iLN-eLN recordings were carried out to investigate. Both groups found eLN-iLN interactions, but quantitative comparisons are difficult. In ten pairs, Huang et al. found no evidence for eLN-iLN connection and one example of a connection in the opposite direction. In contrast, Yaksi and Wilson found that eLNs could frequently excite their paired iLNs through mixed electrical/chemical synapses. The converse interaction was found more rarely and was either in the form of a gap junction or of a chemical synapse. At least two factors may contribute to the discrepancies. First, the recordings of Huang et al. were made in isolated heads, while Yaksi and Wilson's experiments were in intact animals. Second, both groups may have sampled different subpopulations of iLNs although they shared the same electrophysiological characteristics (large soma action potentials, absence of background IPSPs). Choosing that positive evidence wins over absence of evidence, we conclude that interactions do occur between eLNs and iLNs, although their frequency needs to be better estimated. Finally, Yaksi and Wilson performed experiments whose results are consistent with eLN recruitment of glomerular inhibition by iLNs. This result is notable because although lateral excitation tends to increase the odor response of PNs, some PN odor responses can also be decreased, especially as odor concentration increases. The recruitment of iLNs by eLNs could thus explain how lateral excitation could lead to increasing and spreading inhibition.

In summary, the combined work of these two groups builds a convincing

case for the mediation of lateral excitation by cholinergic *krasavietz* LNs. The complementary use of dye coupling by Huang et al. and of genetics by Yaksi and Wilson further bolsters the electrophysiological evidence for widespread (possibly all-to-all) electrical coupling of eLNs and PNs. The diversity of coupling strengths found by both groups explains the observed heterogeneity in the strength of lateral excitation. The mutual electrical coupling of eLNs and the evidence consistent with direct excitation of eLNs by ORNs (Huang et al.) help explain why lateral excitation acts so quickly. The interactions with iLNs found by Yaksi and Wilson provide a mechanism to explain how lateral excitation can induce the spread of inhibition. The picture that emerges from this work is of glomerular channels densely and heterogeneously connected (Figure 1A) via a wide resistive excitatory network, and able to recruit inhibition through its possibly more selective interactions with the iLN population (Figure 1B).

This beautiful work leads to many interesting further questions. Some are specific to this system. For example, what is the detailed nature of interactions between the eLN and iLN networks (connection matrix, Figure 1B)? The pattern of ORN innervation of eLNs also needs to be elucidated, for the evidence provided by Huang et al. is still indirect. The original screen for cholinergic LNs found several *Gal4* lines containing potential eLNs. Hence it is plausible that yet other populations of eLNs exist. The functional consequences of lateral excitation require further elucidation. Could the coupling of this eLN resistive network, for example, be modulated and thereby change transfer gains and response sensitivity? Yaksi and Wilson report that eLNs are sensitive to even very weak odor levels, and Huang et al. show that eLNs are more sensitive than PNs to weak antennal stimulation. These findings imply that eLNs are set up (possibly simply by convergence?) to detect weak odor stimuli. But why are the strengths of PN to eLN coupling heterogeneous? One possibility is that eLNs boost excitation among groups of PNs whose glomeruli tend to be coactivated. Yet, an earlier study (limited to one glomerulus) showed no correlation between

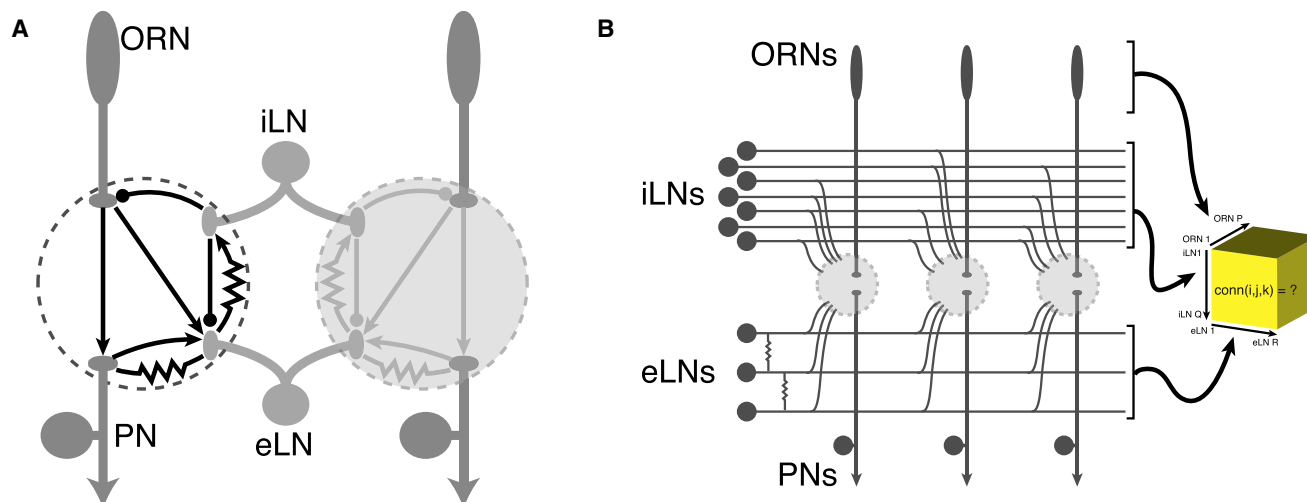


Figure 1. Excitatory LNs Interact with ORNs, PNs, iLNs, and Other eLNs to Form an Excitatory Resistive Network

(A) Qualitative summary of main known connections within glomeruli. Synapses involving eLNs were investigated in the previewed papers. Arrows are excitatory chemical synapses, knobs are inhibitory chemical synapses, resistors are gap junctions (possibly rectifying), and resistors with arrowheads are excitatory chemical synapses coupled with gap junctions in which the proper function of the chemical synapse seems to require the proper formation of the electrical synapse. (B) The excitatory eLN network. Excitatory LNs are densely interconnected by gap junctions. Individual eLNs innervate most if not all glomeruli, while the innervation patterns of iLNs are more diverse. The connectivity matrices describing the interconnection of eLNs, iLNs, and ORNs, have yet to be determined and may vary across individuals.

similarities in glomerular tuning and degree of lateral excitation (Olsen et al., 2007). And given the temporal patterning of eLN responses observed by Huang et al., can the eLNs play a more direct role in the patterning of PN responses? Finally, recent results on iLNs indicate a great diversity of arborization patterns within and across individuals (Chou et al., 2010). While the number of eLNs is much smaller, are those neurons diverse as well, and does their development and final maturation evolve in concert with that of their inhibitory counterparts? The advent of recording techniques that allow simultaneous recordings from large numbers of neurons in the fly, genetic tools providing ever-greater control over neuronal subpopulations, and experimental skills such as those demonstrated by the authors of these two articles is sure to shed light on many of these questions.

Other questions are more general. Given the complexity of even such small sensory networks—the *Drosophila* AL

contains only a few hundred neurons—what can we learn to facilitate the study of ever more complex brains and circuits? These papers should at least sensitize us, if there ever was any need, to the fact that neurons generally do their work while embedded in highly adapted and changeable networks; that the tuning properties of sensory neurons emerge from interactions that occur in space and time, in circuits whose extent and quantitative composition are still generally elusive; and that our intuitions about how such distributed systems behave are still extremely poor. The good news is that much of this challenging and needed work lies ahead of us; the field is increasingly alive and well. These are indeed exciting (and electric) times!

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